Hourly Variation in Total Serum Cholesterol

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During a study of variations in serum cholesterol, we observed striking changes from day to day in certain subjects. In a few individuals the change in serum cholesterol was more than 200 mg. per cent from one morning to the next. In sharp contrast, in other subjects the level of serum cholesterol seemed quite stable. Similar observations have been made by Thomas and Eisenberg. The tendency toward a labile pattern seemed to persist from one year to the next in certain subjects and it therefore seemed desirable to investigate further the rapidity with which changes might occur.

Method and Material

The studies reported here concern hourly variations in total serum cholesterol among individuals in 2 groups of apparently healthy medical students. Group 1 consisted of 5 students who were selected because of a marked lability in serum cholesterol that each had shown during a previous period of observation. These 5 subjects are referred to later as the "labile group." Group 2 consisted of 5 other subjects whose serum cholesterol had seemed quite stable during the previous period of observation. All subjects in each group had participated in earlier studies in which serum cholesterol had been measured before and during the course of semester examinations. At least 12 determinations of serum cholesterol had been made on each subject and these were scattered over the freshman and sophomore years of medicine.

Plan of Study

During Christmas holidays the 5 students constituting the "labile group" were hospitalized from December 29, 1958, until January 2, 1959, to facilitate observation. They were ambulatory with certain limitations imposed by the procedure and were fed a general hospital diet of approximately 2,400 calories. Lacto-ovo-vegetarian food sources were used, and the diet was planned to include 75 Gm. of protein, 100 Gm. of fat, and 300 Gm. of carbohydrate. The participants were told briefly of the general plan to be followed in the study. Polyethylene catheters were placed in a brachial vein and blood samples were collected at hourly intervals from 6 a.m. until 10 p.m. and again at 2 a.m. Between samplings a slow infusion of physiologic salt solution was maintained to prevent clotting of blood within the catheter. The amount of the infusion varied a little from day to day and among the subjects, but averaged 2,400 ml. for each 24 hours.

Subsequent treatment of the 2 groups was as nearly alike as possible except for 3 points. 1. The labile group was studied during the Christmas holidays while the stable group was observed at Easter. 2. Because of technical difficulties hourly blood samples were obtained by repeated venipuncture rather than from the indwelling catheters during the first 4 hours of the experiment with the labile group. 3. The period of exposure in a cold room was omitted for the stable group.

Day 1

The first day was used for control. The subjects were permitted to lounge about their quarters with no disturbance except for the drawing of hourly blood samples.

Day 2

At 2 p.m. all 5 "labile" subjects were placed in a cold room with the temperature at 4 C. They remained in this environment for 45 minutes with minimal clothing to protect them from the cold. On leaving the cold room they were warmed quickly with blankets and a bath.

Day 3

Early on the third day the subjects were told they would be introduced to a situation that would be psychologically stressful. During the morning each subject was brought in turn to the physiology laboratory. He was asked to lie on a cot and leads were attached for simultaneous recording of respiration, electrocardiographic lead II, finger plethysmography, and galvanic skin response. A microphone was then strapped about the subject's neck and rubber-covered ear phones were placed over his ears. In addition to the array of equipment some additional pressure was furnished by the presence of instructors from medicine and physiology who participated in the study.

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As soon as the polygraph record was stable, the delayed auditory feedback procedure was begun. Instructions were read aloud by the subject from a card placed before him:

This test is an index of your ability to adapt to a controlled hospital routine. You will be given a number of cards to read aloud and to answer. The first set of cards will consist of proverbs for you to tell the meaning of in your own words. The second set of cards will consist of medical questions with which you should be thoroughly familiar. After you have interpreted the first three proverbs, you will begin to hear an echo of your voice. It will be your task to explain fully in spite of the echo. Remember to begin each card by reading it aloud.

On completing the interpretation of 15 proverbs under delayed auditory feedback conditions the subject then answered 5 medical questions. The delayed auditory feedback mechanism consisted of a tape recorder modified by the addition of a monitor-head and circuit. The monitor-head was set to provide a 0.30-second delay, an interval of maximum interference with normal speech delivery.

Each such test lasted for approximately 20 minutes. The behavioral reaction varied considerably from one subject to another, although each one showed some characteristic slurring of speech, unfinished words, and retardation of thought processes at the beginning of the auditory feedback.

A few individuals reacted with excessive perspiration, twitching of fingers and toes, completely blocked speech pattern, grossly inadequate interpretation of proverbs, and inability to recall common medical information that was ordinarily well known by the subject. There were no consistent behavioral differences between the 2 groups, and most of the subjects seemed to adapt rather quickly to the auditory feedback simply by a slowing of their responses.

Day 4

This was another control day with no experimentation.

Day 5

At approximately 8:45 a.m. each subject was given 0.6 ml. of epinephrine subcutaneously (Adrenaline Chloride, 1:1000 solution, Parke Davis & Co.), and after 1 hour a second injection of 0.8 ml. was similarly given. Clinical signs of epinephrine effect were clearly evident in each case but none of the subjects was made seriously uncomfortable by this dosage.

Cholesterol Measurement

Total serum cholesterol was measured by the method of Pearson, Stern, and McGavaeck. Each blood sample was tested in duplicate and in any case in which the hourly change was marked, the determination was rechecked along with 1 or 2 samples before and after it. Each value reported in the subsequent data indicates the mean of all determinations on that sample. This method (Pearson, Stern, and McGavaeck) for measuring total serum cholesterol has been used in our laboratory for some time and all determinations were done by the same individuals. Technical error of measurement was rechecked twice during the experiment with use of 20 samples on one occasion and 21 on another. In these determinations technical error equaled 3.24 and 2.14 respectively. This amount of error compares favorably with data reported in the cooperative study of lipoprotein and cholesterol measurements.

Twenty aliquots of pooled serum submitted under code markings and along with other samples showed a range of 271 to 291 mg. per 100 mL, with a mean of 284.2 and a standard deviation of 7.59.

Results

Hourly variations in total cholesterol for subjects in these 2 groups are shown graphically in figures 1 and 2. At least 3 points are evident from these data. 1. Significant changes in the level of serum cholesterol may occur within a few hours in certain individuals. 2. The variation of serum cholesterol is greater and seems more consistently related to certain environmental factors among the subjects in group 1. 3. The mean level of serum cholesterol is higher for group 1, being 252.7 mg. per cent as compared to 188.4 for group 2 (table 1).

Control Day 1

Review of the data from group 1 shows that the level of blood cholesterol declined rather sharply a few hours after the experiment got under way. Before the chemical data were available, we questioned whether this first day could be regarded properly as a control period. In retrospect we doubt that it should be so regarded. All 5 subjects were quite apprehensive initially, and this apprehension was aggravated by technical difficulties. The

*The technical error of measurement is $\sigma e = \sqrt{\sum d^2/2k}$, i.e., the square root of the sum of the squared differences of duplicates divided by twice the number of pairs. This statistic was used on the advice of Dr. Sidney Abraham as a measure of duplicate reproducibility.

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Figure 1

Pattern of hourly changes in level of serum cholesterol for 5 subjects comprising Group 1 (labile). Arrow A indicates time at which subjects entered cold room. Arrow B indicates time at which psychological test was begun. Arrows C and D indicate times at which epinephrine was injected subcutaneously.
Figure 2

Pattern of hourly changes in level of serum cholesterol for 5 subjects comprising Group 2 (stable). Arrow A indicates time when psychological test was begun. Arrows C and D indicate times at which epinephrine was injected subcutaneously.
subjects seemed more relaxed as the day wore on, and it now appears to us that the decline in blood cholesterol somewhat paralleled this lessening of apprehension.

The experiment got under way more smoothly in the case of group 2. No technical difficulties were encountered and initial apprehension was less evident. Hourly changes in cholesterol, among this second group of subjects, were less striking, and no consistent pattern was apparent.

Exposure to Cold

This experiment involved only the subjects in group 1. It can be seen that blood cholesterol rose sharply before the exposure to cold, and it may be significant that these students knew of the intended procedure several hours before entering the cold room. Cholesterol levels dropped rather quickly in 3 of the 5 subjects. In 2 cases there was some secondary rise but we are unable to relate it to any observed events.

Psychological Trial

Changes in the level of serum cholesterol that occurred in conjunction with the delayed auditory feedback procedure are a bit more varied but all the subjects in group 1 showed some striking change. This experience appeared in most instances to be more disturbing than the subjects had expected. This is in contrast to the previous day’s experience with cold in which they commented that the exposure was “not as bad as we had expected.”

Changes in serum cholesterol occurring among the subjects in group 2 were less striking and the patterns were less consistent.

Control Day 2

Differences between the 2 groups of subjects were less evident on this second control day. However, the average serum cholesterol for subjects in group 1 was higher than for those in group 2, and the hourly variation for 2 of the subjects in group 1 was somewhat greater than for the others (table 1). These are the 2 students who had previously shown the greatest fluctuation in serum cholesterol at the times of semester examinations.

Epinephrine

Each subject in our “labile” group showed a prompt rise and fall in blood cholesterol after subcutaneous injection of epinephrine. No such consistent response was seen among the subjects in group 2, and it was therefore arranged to repeat this observation on 3 of the labile subjects. Figure 3 presents the data from this experiment, which was done about 3 months later. The response was similar though a bit less marked than on the first occasion.

Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control mg. %±S.D.*</th>
<th>Cold room mg. %±S.D.</th>
<th>Psychological trial mg. %±S.D.</th>
<th>Control mg. %±S.D.</th>
<th>Epinephrine mg. %±S.D.</th>
<th>Total mg. %±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.P.</td>
<td>251.9±25.9</td>
<td>263.3±42.8</td>
<td>260.3±30.9</td>
<td>256.8±18.4</td>
<td>285.3±36.8</td>
<td>262.9±33.0</td>
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<td>C.R.</td>
<td>238.3±28.7</td>
<td>232.5±30.7</td>
<td>248.9±29.1</td>
<td>259.5±28.3</td>
<td>252.9±27.8</td>
<td>245.9±28.5</td>
</tr>
<tr>
<td>D.I.</td>
<td>254.8±18.0</td>
<td>235.1±25.7</td>
<td>271.8±24.1</td>
<td>252.9±11.8</td>
<td>290.0±29.1</td>
<td>259.6±32.5</td>
</tr>
<tr>
<td>G.B.</td>
<td>219.9±24.4</td>
<td>201.5±19.3</td>
<td>246.7±28.8</td>
<td>227.2±8.0</td>
<td>228.4±15.2</td>
<td>226.6±24.6</td>
</tr>
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<td>W.S.</td>
<td>238.6±21.7</td>
<td>233.2±23.8</td>
<td>298.6±27.3</td>
<td>260.2±5.4</td>
<td>291.2±34.5</td>
<td>267.8±32.2</td>
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<tr>
<td>Group 2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.M.</td>
<td>188.7±11.4</td>
<td>193.7±9.1</td>
<td>205.4±11.1</td>
<td>193.2±12.1</td>
<td>196.3±13.5</td>
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<tr>
<td>E.G.</td>
<td>173.8±8.5</td>
<td>177.1±11.8</td>
<td>193.1±10.1</td>
<td>179.1±13.9</td>
<td>181.1±12.5</td>
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<tr>
<td>H.G.</td>
<td>133.5±8.3</td>
<td>149.2±10.5</td>
<td>154.0±9.6</td>
<td>156.6±6.3</td>
<td>148.5±12.4</td>
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<tr>
<td>B.H.</td>
<td>187.9±10.6</td>
<td>197.3±14.7</td>
<td>200.8±10.9</td>
<td>219.8±12.5</td>
<td>201.1±16.4</td>
<td></td>
</tr>
<tr>
<td>J.M.</td>
<td>209.3±9.3</td>
<td>220.5±14.3</td>
<td>215.0±10.5</td>
<td>219.5±11.2</td>
<td>216.2±12.3</td>
<td></td>
</tr>
</tbody>
</table>

*S.D. = standard deviation.

Technical error of measurement (σ e) = 2.25.
Figure 3
Re-check of hourly changes in serum cholesterol following subcutaneous injection of epinephrine in 3 subjects of Group I. Arrows A and B indicate times at which epinephrine was given.

Discussion
Diurnal variations in serum cholesterol have been studied by several investigators. The majority\textsuperscript{6-9} reported little change, although McEachern and Gilmour\textsuperscript{10} found wide variations in the 5-hour cholesterol curves of certain individuals. They concluded, on this account, that single or haphazard measurements of blood cholesterol were of doubtful value.

The variations of serum cholesterol at longer intervals have been studied more extensively.\textsuperscript{11-14} Schube\textsuperscript{13} reported that blood cholesterol fluctuates differently among individuals and some observers\textsuperscript{15, 16} have reported that the serum cholesterol of patients with coronary arteriosclerosis is inconstant and fluctuates widely in contrast to normal persons in whom the level is relatively stable.

The influence of stress on serum cholesterol is complicated by the difficulty in defining and limiting a stressful experience. Our data suggest that the anticipation of certain events may be related chronologically to changes in the level of serum cholesterol as easily as the events themselves. It appears to us that sham exposure will be necessary in order to clarify this point. Mann and White\textsuperscript{17} reported that the physiological response to stress is a reduction in total serum cholesterol with a disproportionate fall in the esterified fraction. Others\textsuperscript{1, 18, 19} believed that certain forms of emotional stress are usually associated with a sudden increase in serum cholesterol. The experiments on which these differing views are based are so dissimilar that there might be several reasons for the apparently differing response. The rapid and variable changes in serum cholesterol in our subjects suggest that the nature of a stressful experience, the timing of cholesterol determinations, and the preselection of experimental subjects all might influence such results.

Preselection of subjects is thought to account for the striking and rather consistent cholesterol patterns in our group 1. It should be emphasized that each subject participating in this study of hourly changes was selected because of apparent lability or stability of his blood cholesterol during previous observations which extended over a period of 2 years. Those showing the most striking changes in cholesterol at the time of semester examinations were assigned to group 1. For contrast, the subjects in group 2 were those whose cholesterol levels had shown relatively little fluctuation during the same period of time. It now appears that we might have been able to distinguish between these 2 groups of students by noting the changes in serum cholesterol after injection of epinephrine. We are not certain that this would be true of a larger group or even of these same individuals over a longer period of time. It is planned to repeat such tests with these same subjects and also to extend the studies to include a larger number of persons.

These observations support the view that the level of serum cholesterol is more variable in some individuals than in others. They also indicate that remarkable changes may occur with greater rapidity than we had supposed. The data suggest that certain situations appearing potentially to be stressful may induce rather striking changes in serum cholesterol within a few hours in selected individuals. It remains to be seen whether lability or stability will continue to be characteristic.
of these persons. We are unable at this time to relate these patterns to any evidence of vascular or other disease and can only speculate about the mechanisms by which such rapid changes may occur.

Summary

Evidence is presented to indicate that the level of serum cholesterol in certain individuals may vary widely within a matter of hours.

The data suggest also that a rapid fluctuation of the serum cholesterol level may be induced in some persons by modifying certain aspects of their environment.

Varying lability of the level of serum cholesterol and the differing response of individuals to certain environmental factors require that careful attention be given to the sampling methods used in research.

Summario in Interlingua

Es presentate observationes que indica que le nivello del cholesterol serial e certe subjectos pote variar extensemente intra alien horas.

Le observationes etiam suggere que un fluctuation rapide del nivello de cholesterol serial pote esser inducita in certe subjectos per le modification de certe aspectos de lor ambiente.

Varie grados de labilitate del nivello de cholesterol serial e le variate di responsas del parte de varie subjectos a certe factores del ambiente demanda que le plus circumspecte attention es prestata al methodos de specimineg useate in investigationes scientifce.

References
